DATA EVALUATION RECORD
HONEY BEE - ACUTE CONTACT & ORAL LD_{50} TEST
§141-1

1. CHEMICAL: Novaluron PC Code No.: 124002

2. TEST MATERIAL: "RIMON" Technical Purity: 99.3%

3. CITATION:

Author: Gray, A.P.

Title: "RIMON" Technical Acute Toxicity to Honey Bees (*Apis mellifera*)

Study Completion Date: 35802

Laboratory: Huntingdon Life Sciences, Ltd.
P.O. Box 2, Huntingdon
Cambridgeshire, England

Sponsor: Makhteshim Chemical Works Ltd.
P.O.B. 60
Beer-Shave, Israel

Laboratory Report ID: MAK 433/973447

DP Barcode: D285479

MRID No.: 45638220

4. REVIEWED BY: Rebecca Bryan, Staff Scientist, Dynamac Corporation

Signature: Rebecca Bryan **Date:** 4/1/03

APPROVED BY: Dana Worcester, Staff Scientist, Dynamac Corporation

Signature: Dana Worcester **Date:** 4/1/03

5. APPROVED BY: Bill Evans

Signature: Bill Evans **Date:** 11/3/03

6. STUDY PARAMETERS:

Scientific Name of Test Organism: *Apis mellifera*

Age or Size of Test Organism at Test Initiation: Worker honey bees, age not specified

Type of Concentrations: Nominal

Definitive Study Duration: 48 hours

7. CONCLUSIONS:

The honey bee, *Apis mellifera* L., was exposed to Novaluron ("RIMON" Technical) for 48 hours in both oral and contact toxicity tests. In the oral and contact tests, the nominal test concentration was 100 µg/bee. By 48 hours in the oral test, 6.7% mortality was observed in the 100 µg/bee treatment group, compared to 1.7% negative control mortality and 5.0% solvent control mortality. By 48 hours in the contact test, 6.7% mortality was observed in the 100 µg/bee treatment group, compared to 1.7% negative control mortality and 3.3% solvent control mortality.

The LD_{50} value for the oral test was >100 µg/bee. The LD_{50} value for the contact test was >100 µg/bee. As a result, Novaluron is categorized as practically nontoxic to honeybees on both an acute oral and contact basis.

This acute contact study is classified as Core. This study is scientifically sound and it satisfies the EFED concerning the guideline requirements for a contact toxicity test with honey bees (Subdivision L, §141-1 or 850.3020). **The acute oral study is scientifically sound and is classified as Supplemental.**

Reported Statistical Results - Oral Test:

LD_{50}: >100 µg/bee 95% C.I.: N/A
NOEC: 100 µg/bee Probit Slope: N/A

Reported Statistical Results - Contact Test:

LD_{50}: >100 µg/bee 95% C.I.: N/A
NOEC: 100 µg/bee Probit Slope: N/A

8. ADEQUACY OF THE STUDY:

A. Classification: This acute contact study is classified as Core. This study is scientifically sound and it satisfies the EFED concerning the guideline requirements for a contact toxicity test with honey bees (Subdivision L, §141-1 or 850.3020). The acute oral study is scientifically sound and is classified as Supplemental.

B. Rationale: This acute oral study is scientifically sound and is classified as Supplemental because the study is a non-guideline study and does not fulfill an OPP guideline requirement.

C. Repairability: N/A

9. GUIDELINE DEVIATIONS:

1. The age of the worker honey bees were not reported.

10. SUBMISSION PURPOSE: This study was submitted to provide data on the acute oral and contact toxicity of Novaluron to honeybees for the purpose of chemical registration.

11. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species: Species of concern (*Apis mellifera, Megachile rotundata,* or *Nomia melanderi*)	*Apis mellifera*
Age at beginning of test:	Worker honey bees, age not specified.
Supplier:	Mr. R. Baker, St Ives, Cambridgeshire, UK
All bees from the same source?	Yes

B. Test System

Guideline Criteria	Reported Information
Cage size adequate?	Stainless steel wire mesh cages (11.5 cm tall x 4.0 cm diameter).
Lighting:	Continuous darkness
Temperature:	24-25°C
Relative humidity:	52-63%

C. Test Design

Guideline Criteria	Reported Information
Range finding test?	The definitive limit test was based on results of contact and oral range finding studies. Results not reported.
Reference toxicant test?	Dimethoate
Method of administration:	Oral test: The test solution (500 µL) containing acetone was diluted to 10 mL with a 50% sucrose solution. 200 µL of test solution was provided per cage. Contact test: The test substance was dissolved in acetone, and 1 µL of the test solution was applied to the ventral thorax of each bee using a microapplicator.
Nominal doses:	Oral test: 100 µg/bee Contact test: 100 µg/bee

Guideline Criteria	Reported Information
Controls: Negative control and/or diluent/solvent control	Oral test: negative (untreated) and solvent (50% sucrose solution mixed with acetone). Contact test: negative (untreated) and solvent (acetone)
Number of colonies per group:	6 replicates; 10 bees/replicate
Solvent: The following solvents: acetone, dimethylformamide, triethylene glycol, methanol, ethanol.	Acetone, 100 µg/µL
Feeding:	Oral test: After treated solutions were consumed (four and a half hours), bees were supplied with untreated 50% sucrose solution containing acetone, *ad libitum.* Contact test: A 50% sucrose solution was provided *ad libitum.*
Observations period:	48 hours

12. REPORTED RESULTS:

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Control performance:	Oral test: 1.7% negative control mortality in and 5.0% solvent control mortality by 48 hours. Contact test: 1.7% negative control mortality in and 3.3% solvent control mortality by 48 hours.

Guideline Criteria	Reported Information
Raw data included:	Data were provided.
Signs of toxicity (if any) were described?	No sublethal effects were observed.

Mortality - Oral Test

Dosage (µg/bee)	No. of bees	Rep.	Cumulative Number of Dead	
			Hour of Study	
			24	48
Test Substance (Novaluron):				
Negative control	1e+11	123456	0	1000
Solvent control (Acetone)	1e+11	123456	11001	11001
100	1e+11	123456	20011	20011
Toxic Standard (Dimethoate):				
Negative control	101010	123	10	10
Solvent control (Acetone)	101010	123	101	101
0.04	101010	123	211	212
0.16	101010	123	656	656
0.64	101010	123	10108	10109

Observations: By 48 hours, 6.7% mortality was observed in the 100 µg/bee treatment group, compared to 1.7% negative control mortality and 5.0% solvent control mortality.

Mortality - Contact Test

Dosage (µg/bee)	No. of bees	Rep.	Cumulative Number of Dead	
			Hour of Study	
			24	48
Test Substance (Novaluron):				
Negative control	1e+11	123456	0	1000
Solvent control (Acetone)	1e+11	123456	100000	100010
100	1e+11	123456	10011	10111
Toxic Standard (Dimethoate):				
Negative control	101010	123	10	10
Solvent control (Acetone)	101010	123	110	110
0.04	101010	123	500	500
0.16	101010	123	456	457
0.64	101010	123	10910	101010

Observations: By 48 hours, 6.7% mortality was observed in the 100 µg/bee treatment group, compared to 1.7% negative control mortality and 3.3% solvent control mortality.

Statistical method: The LD_{50} values were estimated based on mortality and sublethal effects data in the oral and contact toxicity tests.

Reported Statistical Results - Oral Test:

LD_{50}: >100 µg/bee 95% C.I.: N/A
NOEC: 100 µg/bee Probit Slope: N/A

Reported Statistical Results - Contact Test:

LD_{50}: >100 µg/bee 95% C.I.: N/A

NOEC: 100 µg/bee Probit Slope: N/A

13. VERIFICATION OF STATISTICAL RESULTS:

Statistical method: The NOEC value was determined by comparing treatment to control data using a Student's t-test for both the oral and contact toxicity tests. Because mortality did not exceed 50%, the LD_{50} could be visually estimated.

Results - Oral Test:

LD_{50}: >100 µg/bee 95% C.I.: N/A
NOEC: 100 µg/bee Probit Slope: N/A

Results - Contact Test:

LD_{50}: >100 µg/bee 95% C.I.: N/A
NOEC: 100 µg/bee Probit Slope: N/A

14. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to the study authors. **The LD_{50} value for the oral test was >100 µg/bee. The LD_{50} value for the contact test was >100 µg/bee. As a result, Novaluron is categorized as practically nontoxic to honeybees on both an acute oral and contact basis.**

For the oral toxicity test, the 48-hour LD_{50} of the toxic standard, dimethoate, was 0.14 µg/bee. For the contact toxicity test, the 48-hour LD_{50} of the toxic standard, dimethoate, was 0.15 µg/bee.

15. REFERENCES:

Gough, H.J., McIndoe, E.C. & Lewis, G.B. (1994) The use of dimethoate as a reference compound in laboratory acute toxicity tests on honey bees (*Apis mellifera* L.) 1981-1992. *Journal of Apricultural Research*, 33(2): 119-125.

Thompson, W.R. & Weil, C.S., (1952) On the construction of tables for moving average interpolation *Biometrics*, 8: 51-54.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

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Survival oral test
        Standard Two-Sample t-Test

data:  solvent control: V1 in DS1 , and 100 ug/bee: V2 in DS1
t = 0.4152, df = 10, p-value = 0.6867
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
  -7.276782  10.610115
sample estimates:
mean of solvent control: 95
mean of 100 ug/bee: 93.33333


Survival contact test
        Standard Two-Sample t-Test

data:  solvent control: V3 in DS1 , and 100 ug/bee: V4 in DS1
t = 1.118, df = 10, p-value = 0.2897
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -3.309693  9.976360
sample estimates:
mean of solvent control: 96.66667
mean of 100 ug/bee: 93.33333
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